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Calcium Signaling in Cardiac Myocytes

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Calcium (Ca^{2+}) is a critical regulator of cardiac myocyte function. Principally, Ca^{2+} is the link between the electrical signals that pervade the heart and contraction of the myocytes to propel blood. In addition, Ca^{2+} controls numerous other myocyte activities, including gene transcription. Cardiac Ca^{2+} signaling essentially relies on a few critical molecular players—ryanodine receptors, voltage-operated Ca^{2+} channels, and Ca^{2+} pumps/transporters. These moieties are responsible for generating Ca^{2+} signals upon cellular depolarization, recovery of Ca^{2+} signals following cellular contraction, and setting basal conditions. Whereas these are the central players underlying cardiac Ca^{2+} fluxes, networks of signaling mechanisms and accessory proteins impart complex regulation on cardiac Ca^{2+} signals. Subtle changes in components of the cardiac Ca^{2+} signaling machinery, albeit through mutation, disease, or chronic alteration of hemodynamic demand, can have profound consequences for the function and phenotype of myocytes. Here, we discuss mechanisms underlying Ca^{2+} signaling in ventricular and atrial myocytes. In particular, we describe the roles and regulation of key participants involved in Ca^{2+} signal generation and reversal.

OVERVIEW OF THE CARDIAC CYCLE

The mammalian heart is a complex organ consisting of four chambers—the left and right atria and the left and right ventricles. Through a highly coordinated series of events, the muscular heart pumps blood through the pulmonary and systemic vasculature (Fukuta and Little 2008). During diastole, all four chambers are relaxed. Systole is initiated by propagation of a depolarizing action potential from the sino-atrial node located in the apex of the right atrium, through the right and then the left atrium. This depolarization induces contraction of these chambers, forcing blood into the ventricles. On reaching the atrioventricular (AV) node, the depolarization pauses for a short time period (0.1 s in humans) to ensure completion of atrial systole. Importantly, the AV node acts as an electrical insulator between the atria and ventricles. The AV node prevents the transfer of aberrant contraction patterns to the ventricles, such as the spontaneous electrical activity occurring during atrial fibrillation. The lower portion of the AV node is designated the bundle of His, which then splits into the left and right branches, allowing activation of the left and right ventricles,

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respectively. These branches give rise to thin filaments called Purkinje fibers, composed of noncontractile cells that distribute the action potential to ventricular myocytes and enable the heart to contract in a coordinated fashion. Transduction of the depolarization signal through the His-Purkinje system causes ventricular systole. The contraction wave, traveling up from the ventricular base, expels blood into the pulmonary artery then on to the lungs, or through the aorta into the arterial system. Retrograde flow of blood is prevented by valves between the atria and ventricles.

As indicated above, the SA node situated at the apex of the right atrium is responsible for initiation of the cardiac action potential. At rest, the membrane potential starts around −70 mV (V_m) and slowly depolarizes until an action potential is triggered. Ca^{2+} signals may play a key role in action potential generation, although there is considerable debate regarding the major mechanisms controlling the rate of SA node depolarization (see Lakatta and DiFrancesco 2009). One primary component of SA node depolarization is known as I_f (f stands for funny) (Brown et al. 1979), an ion current mediated by hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels (DiFrancesco 1993). Because this current is triggered by hyperpolarization, it is activated at the start of diastole and slowly declines throughout the pacemaker period. HCN channels are relatively nonselective, and they therefore generate an inward current depolarizing V_m toward the threshold for firing an action potential. Intracellular Ca^{2+} cycling has also been proposed to act as a primary regulator of SA node depolarization. Imaging SA node cells reveals spontaneous elementary Ca^{2+} signals known as Ca^{2+} sparks arising from the SR and preceding action potential generation (Huser et al. 2000). Ca^{2+} sparks reflect the concerted opening of a cluster of RyRs. The Ca^{2+} sparks activate sodium/calcium exchange (NCX), which promotes membrane depolarization because three Na^+ ions enter for each Ca^{2+} ion that leaves. T-type Ca^{2+} channels ("transient current;" Ca_v3) may also provide a source of Ca^{2+} for triggering Ca^{2+} sparks (Bogdanov et al. 2001; Berridge 2003). When V_m reaches a critical threshold (−40 to −50 mV), plasma membrane L-type Ca^{2+} channels are opened ($I_{Ca,L}$), allowing a large influx of Ca^{2+} into the cytosol and increasing the membrane potential to ∼+10 mV. It is this depolarization signal that is transmitted from the SA node through the cardiac conduction system, culminating in cardiac myocyte contraction. Within the SA node cells, $I_{Ca,L}$ activates an outward potassium current (I_K) which hyperpolarizes the membrane and curtails the action potential. The hyperpolarization leads to activation of I_f, T-type Ca^{2+} channels and Ca^{2+} sparks, to begin the next conduction cycle.

EXCITATION-CONTRACTION COUPLING (EC-COUPLING)

EC-coupling is the process pairing myocyte depolarization with mechanical contraction. Ca^{2+} is the critical intermediary (Bers 2008). Indeed, since Ringer's experiments more than a century ago, Ca^{2+} has been known to be an essential mediator of this process (Ringer 1883). As the action potential sweeps over the heart, the plasma membrane (sarcolemma) of each myocyte becomes depolarized (∼−90 mV to ∼+20 mV) thereby causing concerted opening of L-type VOCCs ("long-lasting current;" $Ca_v1.2$). Ca^{2+} flows via the VOCCs into a restricted space between the sarcolemma and the underlying sarcoplasmic reticulum (SR) known as the "junctional zone" or "dyadic cleft." The accumulation of Ca^{2+} ions during an action potential increases the Ca^{2+} concentration within this microdomain from ∼100 nM to ∼10 μM. This elementary Ca^{2+} influx signal, derived from the activation of VOCCs is known as a "Ca^{2+} sparklet" (Fig. 1) (Wang et al. 2001). The distribution of Ca^{2+} sparklet magnitudes suggests that one or several VOCCs can give rise to such signals within myocytes (Cheng and Wang 2002).

Ca^{2+} sparklets themselves are not adequate to cause substantial contraction. However, they are sufficient to induce opening of RyRs (type 2 RyRs) on the closely apposed SR, via a process known as "Ca^{2+}-induced Ca^{2+} release"

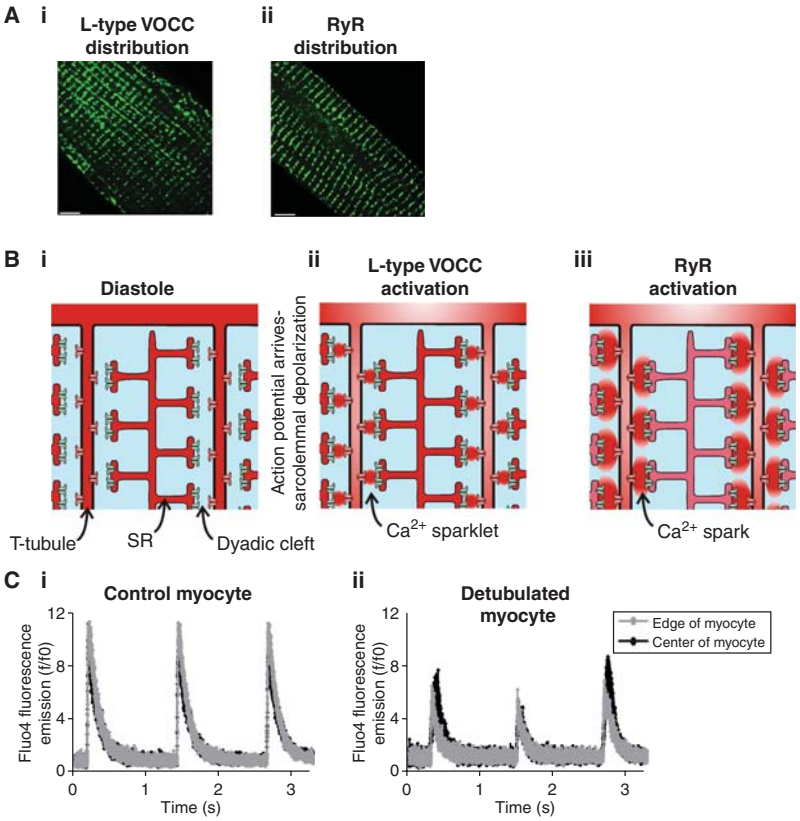

Figure 1. Excitation contraction coupling in ventricular myocytes. Panel *A* illustrates the distribution of L-type VOCCs (*Ai*) and type 2 RyRs (*Aii*) in a section of a ventricular myocyte. The distributions of these proteins are essentially overlapping at the level of the light microscope. Panel *B* is a cartoon sequence of events leading to the generation of a Ca^{2+} signal within a ventricular myocyte. A small section of a ventricular myocyte is depicted with two T-tubule projections (T-tubule spacing ~1.8 μm). During the diastolic phase (*Bi*), the L-type VOCCs (red channels on the T-tubule membranes) and RyRs (blue channels on SR membrane) are silent. Arrival of the action potential causes depolarization of the sarcolemma and activation of the L-type VOCCs thereby generating "Ca^{2+} sparklets" (*Bii*). The Ca^{2+} sparklets trigger activation of the RyRs thereby producing "Ca^{2+} sparks" (*Biii*). Panel *Ci* depicts the consistent, global Ca^{2+} responses observed in an electrically paced ventricular myocyte. The black and gray traces indicate the Ca^{2+} concentration (measured with fluo4) at the center and edge of the myocyte. The profile of the Ca^{2+} signal was essentially the same in both locations. Panel *Cii* illustrates what happens in a ventricular myocyte following detubulation (using formamide treatment). Detubulation decreases the amplitude of systolic Ca^{2+} transients, and provokes spatial heterogeneity of the resultant Ca^{2+} signals. The black and gray traces indicate the Ca^{2+} concentration at the center and edge of the myocyte. Whereas the Ca^{2+} responses in the edge of the myocyte were reasonably consistent, the signals in the center of the cell showed beat-to-beat variation in amplitude. Such Ca^{2+} signal alternans are a potential cause of cardiac arrhythmia.

(CICR). Seminal studies in the 1970s showed that RyR activity is dependent on cytosolic Ca^{2+} levels, with low concentrations (1–10 μM) being activatory and high concentrations (>10 μM) inhibiting the channel (Fabiato and Fabiato 1972; Fabiato 1983). The concentration of Ca^{2+} at the dyadic cleft following activation of L-type Ca^{2+} channels falls within the range of that required for channel activation, thus facilitating CICR.

The activation of a cluster of RyRs, and consequent mobilization of Ca^{2+} from the SR, produces an elementary Ca^{2+} release signal know as a "Ca^{2+} spark" (Fig. 1) (Cheng and

Lederer 2008). As with Ca^{2+} sparklets, Ca^{2+} spark magnitudes can vary, indicating that different numbers of RyRs participate in their generation. Release of Ca^{2+} through the RyRs increases the concentration of Ca^{2+} to $>100\ \mu M$ in the dyadic cleft. It is estimated that $\sim$25 L-type Ca^{2+} channels and 100 RyRs are closely associated within the dyadic cleft to form a "couplon" (Bers and Guo 2005). Ca^{2+} ions diffuse out of the cleft to engage the contractile machinery, thereby promoting cell shortening to provide the force for pumping blood. During a single action potential, thousands of Ca^{2+} spark sites are simultaneously activated by their corresponding Ca^{2+} sparklet triggers (Cheng and Lederer 2008). Diffusion of Ca^{2+} ions, and their subsequent spatial and temporal summation, produces an average global Ca^{2+} increase of 500 nM to $\sim 1\ \mu M$. Troponin C (TnC), the Ca^{2+}-binding component of the contractile filaments, is sensitive to Ca^{2+} concentrations over that range thereby allowing coupling of the AP-mediated Ca^{2+} transient and contraction. At the end of an action potential Ca^{2+} transients are rapidly terminated, and the cells return to resting diastolic levels in preparation for the next depolarization.

In addition to L-type VOCCs, contractile cardiac myocytes express a T-type current (Ca_v3 family), which is so named because of its transient nature. As described in the section on pacemaking above, this current is activated at a more negative membrane potential than the L-type (Nowycky et al. 1985). Although $I_{Ca,T}$ plays a role in depolarizing SA node cells, under normal physiological situations its role is negligible within ventricular and atrial cardiac myocytes as most Ca^{2+} enters through the L-type channels. However, T-type VOCC expression is up-regulated during cardiac hypertrophy, and they may provide a critical Ca^{2+} signal to drive hypertrophic remodeling. For example, $Ca_v3.2$ knockout mice did not display cardiac hypertrophy in response to pressure overload or angiotensin II (Chiang et al. 2009).

Skeletal muscle EC-coupling is also mediated by elevations in intracellular Ca^{2+}, and has some similarities to the cardiac scheme described above. The key difference in this tissue is Ca^{2+} sparklets do not trigger Ca^{2+} release from the SR. Rather, L-type VOCCs ($Ca_v1.1$) on the sarcolemma have a direct physical interaction with RyRs (type 1 RyRs) on underlying SR forming the triadic junction (Rios and Brum 1987; Block et al. 1988). Depolarization of the sarcolemma induces a conformational change in the VOCCs, which allosterically activates the RyRs.

REGULATION OF L-TYPE VOCCs

The channels and homeostatic processes underlying Ca^{2+} release and Ca^{2+} clearance are subject to regulation by multiple signaling pathways. These signaling pathways can rapidly alter the amplitude and/or spatial properties of myocyte Ca^{2+} signaling to acutely modulate cardiac output. For example, L-type VOCCs are subject to regulation by cAMP-dependent kinase (protein kinase A; PKA) downstream from β-adrenergic receptor stimulation, e.g., during the fight or flight response. PKA phosphorylation increases channel activity, thereby contributing to the increased cardiac contraction (positive inotropic response) evoked by β-adrenergic stimuli. PKA-mediated phosphorylation increases opening of the VOCC by a twofold mechanism—it increases both the number of channels in an activatable state, and their activation probability (Catterall 2000). PKA phosphorylates Ser-1928 in the amino terminal of the α_1 subunit (Perets et al. 1996), in addition to residues on the β_2 subunit (Curtis and Catterall 1985; Gerhardstein et al. 1999). Rapid dephosphorylation of the channel is provided by the Ser/Thr phosphatases 1 and 2A (PP1 and PP2A, respectively) (Kamp and Hell 2000).

L-type VOCCs are also phosphorylated by protein kinase C (PKC) in response to activation of G_q-coupled receptors, e.g., α_1-adrenergic, endothelin, and angiotensin II receptors. The targets for PKC phosphorylation are proposed to be two Thr residues in the amino terminal of the α_1 subunit (Shistik et al. 1998; McHugh et al. 2000). The effects of PKC phosphorylation are less clear than for PKA phosphorylation. It is proposed that the effect may

be reliant on the particular PKC isoform activated, the expression of which varies in a complex developmental, species-dependent, and disease-regulated manner in the heart. Additional regulation of the L-type Ca^{2+} channel is provided by cGMP-dependent protein kinase G (PKG), which has been shown to have an inhibitory effect, thus opposing the effects of PKA (Hartzell and Fischmeister 1986; Abi-Gerges et al. 2001).

Inactivation of the L-type Ca^{2+} channel is mediated by both membrane repolarization and by Ca^{2+} itself (Ca^{2+}-dependent inactivation, CDI). The latter acts as a negative feedback loop, and is thought to be the more important of the two mechanisms (Lee et al. 1985). CDI is controlled by calmodulin (CaM), which is constitutively bound to the channel (Peterson et al. 1999; Qin et al. 1999). The CaM binding site is a canonical "IQ" CaM-binding motif within the carboxyl terminus of the α_1 subunit that binds the Ca^{2+}-free form of CaM (apo-CaM) (Rhoads and Friedberg 1997; Zuhlke and Reuter 1998). Ca^{2+} entering through L-type channels during EC-coupling binds to apo-CaM to form Ca-CaM, which inactivates the channel (Tang et al. 2003). Interestingly, Ca-CaM can also enhance Ca^{2+} entry through L-type Ca^{2+} channels by Ca^{2+}-dependent facilitation (CDF). CDF is also dependent on the IQ motif in the cytoplasmic tail of the α_{1C} subunit (Zuhlke et al. 1999). This capacity for dual regulation is caused by the presence of both high and low affinity Ca^{2+} binding sites (EF-hands) within CaM, in the carboxyl and amino terminal lobes, respectively. It is believed that CDI depends on Ca^{2+} bound to the amino terminal EF-hands, whereas CDF depends on the carboxyl terminal EF hands (DeMaria et al. 2001). Subsequently, it was revealed that the two lobes of CaM can detect Ca^{2+} arising from distinct sources—the high affinity carboxyl terminal site sensing local Ca^{2+} arising within the nanodomain of the channel mouth, whereas the low affinity amino terminal lobe detects global Ca^{2+} signals (Tadross et al. 2008). It therefore appears that Ca^{2+} binding to apo-CaM at the carboxyl terminus of L-type VOCCs potentiates Ca^{2+} entry once a Ca^{2+} sparklet is forming. However, once global Ca^{2+} is elevated, Ca^{2+} also binds to the amino terminal lobe of CaM leading to CDI and termination of Ca^{2+} entry.

Additional modification of $I_{Ca,L}$ is provided by Ca^{2+}/CaM-dependent kinase II (CaMKII), which potentiates the influx of Ca^{2+} (Anderson et al. 1994; Xiao et al. 1994; Yuan and Bers 1994; Wu et al. 2001b). CaMKII interacts with and phosphorylates the carboxyl terminal of the α_1 subunit (Hudmon et al. 2005). CaMKII remains tightly bound to the channel even in the absence of Ca^{2+}, although it is only active when it has Ca-CaM bound. Importantly, Ca-CaM can remain bound to CaMKII, and the kinase active, even after global Ca^{2+} has declined, thereby allowing CaMKII to act as a detector of Ca^{2+} spike frequency (Hudmon et al. 2005).

REGULATION OF RyRs AND CICR

There are three mammalian RyR isoforms (RyR1–3). RyR2 is predominant in cardiac myocytes, with significantly lesser amounts of RyR3. The RyR channel is a large homotetrameric assembly of ~2 megadaltons (each subunit has a molecular mass of ~560 kDa) (Lanner et al. 2010). Structural studies have revealed four-fold symmetry, with a four-leaf clover or mushroom morphology formed by the transmembrane domain and bulky cytoplasmic domain (Anderson et al. 1989; Serysheva 2004). Binding of Ca^{2+} is proposed to cause conformational changes that evoke channel gating in a mechanism similar to that of a camera iris, with twisting of the transmembrane regions opening the ion pore (Serysheva et al. 1999).

RyRs are bound by a multitude of accessory proteins, comprising a macromolecular signaling complex. These interactions determine the efficiency and specificity of signaling to and from RyRs, and between other signal transduction cascades. Moreover, scaffolding of proteins to RyRs recruits and concentrates important regulatory proteins in the junctional zone where they are ideally located to modulate

and/or be regulated by EC-coupling. These interactions occur on both the lumenal and cytosolic face of the RyR (Lanner et al. 2010). In the lumen of the SR, RyRs interact with calsequestrin (CSQ), the major Ca^{2+} binding/storage protein of muscle. CSQ is a low-affinity Ca^{2+} storage protein that maintains the SR lumenal free Ca^{2+} concentration between 100–500 μM (Yano and Zarain-Herzberg 1994; Berridge 2002). The critical role of CSQ in Ca^{2+} storage was shown by the increase or decrease in SR Ca^{2+} load observed in experiments in which CSQ expression was enhanced or suppressed, respectively (Terentyev et al. 2003). In addition to acting as the major Ca^{2+} storage protein in cardiac muscle, CSQ also regulates RyR channel activity (Prins and Michalak 2011). CSQ reversibly changes between monomeric to oligomeric forms in response to changes in luminal Ca^{2+} concentration. It has been suggested that CSQ oligomers are present when the SR is replete, and that these mainly serve to buffer Ca^{2+}. However, when RyRs open and SR luminal Ca^{2+} declines, Ca^{2+} unbinds from calsequestrin and the oligomeric protein dissociates. The calsequestrin monomers bind to RyRs (via a protein intermediate called triadin) and inhibit channel activity. This is an important component of the mechanisms that terminate Ca^{2+} release during each heartbeat (Gyorke et al. 2009).

The key role of CSQ in regulating Ca^{2+} storage and RyR function is highlighted by a pathological condition known as catecholaminergic polymorphic ventricular tachycardia (CPVT) that is observed in patients with CSQ mutations. CPVT is a life-threatening form of cardiac dysrhythmia typically brought about by emotional or physical stress. Recessive CSQ mutations are found in ~3% of CPVT patients (Katz et al. 2009). The effects of some CPVT-inducing CSQ mutations on calcium fluxes in cardiac myocytes are known. For example, mutation of the aspartate to histidine at residue 307 in CSQ (CSQ^{D307H}) causes decreased SR Ca^{2+} storage and release, and increases the frequency of delayed afterdepolarizations (DADs; spontaneous electrical depolarization of cardiac myocyte independent of the SA node-evoked AP) (Viatchenko-Karpinski et al. 2004). Another mutation, arginine to glutamic acid at residue 33 (CSQ^{R33Q}), decreases the interaction between the RyR and CSQ, resulting in abnormal regulation of the RyR by lumenal Ca^{2+} and increased Ca^{2+} release (Terentyev et al. 2006). A substantial proportion of CPVT patients (~50%) express mutated RyRs that have altered association with accessory proteins.

CaM is an important regulator of the RyR in cardiac myocytes, both in its Ca^{2+} bound and Ca^{2+} free form (Ca-CaM and apo-CaM, respectively). The binding site for CaM on the RyR was mapped to the carboxyl terminal of the receptor by site-directed mutagenesis (Porter Moore et al. 1999a; Porter Moore et al. 1999b), in agreement with cryo-EM studies (Wagenknecht et al. 1997). Binding of CaM decreases Ca^{2+} efflux through these channels (Meissner and Henderson 1987). This is thought to be facilitated by a reduction in the opening probability and in the Ca^{2+}-dependent activation of the channel (Balshaw et al. 2001).

Further Ca^{2+}-dependent modification of RyRs is provided by CaMKII-dependent phosphorylation. Sequence analysis revealed six consensus phosphorylation sites in the RyR (Zucchi and Ronca-Testoni 1997). Both Ser-2809 (Witcher et al. 1991) and Ser-2815 (Wehrens et al. 2004) have been shown to be crucial for CaMKII-dependent phosphorylation. CaMKII phosphorylation increases RyR activity (Witcher et al. 1991; Wehrens et al. 2004), although some studies have contested this idea (Lokuta et al. 1995; Wu et al. 2001a). Phosphorylation by CaMKII is emerging as the dominant mode of regulation of RyR activity during adrenergic stimulation and in the greater contractility associated with increased frequency of myocyte contraction (Wu et al. 2009; Grimm and Brown 2010).

Ca^{2+} release through RyRs is regulated by interaction with FK binding proteins (FKBPs), named because of their binding of the immunosuppressant drug FK506. Cardiac myocytes express two isoforms of the 12 kDa FKBP, namely FKBP12 and FKBP12.6 (also known as calstabin1 and calstabin2, respectively). The

cardiac RyR2 binds FKBP12.6 with a higher affinity (Timerman et al. 1996; Jeyakumar et al. 2001). FKBP12.6 binding stabilizes the coordinated gating of RyR subunits within a tetramer, thereby enabling channels to transition between the fully closed and the fully open state, while also shifting the Ca^{2+} dependence of channel opening to a higher Ca^{2+} concentration (Bers 2004). CPVT-inducing mutations within the type 2 RyR channel decrease the association between the receptor and FKBP12.6, although only under conditions of β-adrenergic stimulation. FKBP12.6 dissociation may lead to increased Ca^{2+} release from the SR (Wehrens et al. 2003).

RyRs can also be phosphorylated by PKA, which is tethered by an A kinase anchoring protein (mAKAP) (Marx et al. 2000). PKA-dependent phosphorylation of the receptor was reported to increase its responsiveness to Ca^{2+} (Valdivia et al. 1995), although the precise consequences of PKA-dependent phosphorylation have been controversial. A widely discussed model proposed that PKA phosphorylation of RyRs causes dissociation of FKBP12.6, thereby leading to increased probability of channel opening (Marx et al. 2000; Wehrens et al. 2003). This model of RyR regulation would not only be relevant physiologically, coupling β-adrenergic stimulation with enhanced Ca^{2+} release, but would also be important in disease conditions in which elevated PKA phosphorylation had been reported to decrease FKBP12.6-RyR associations and result in increased spontaneous diastolic RyR activity and DADs. However, the dependence of FKBP12.6 association with the RyR on PKA phosphorylation has been much disputed (Xiao et al. 2007). Indeed, a recent report provides substantial evidence that the association of FKBP12.6 interaction with the RyR is insensitive to the degree of PKA phosphorylation (Guo et al. 2010).

PKA may also regulate RyR activity by phosphorylation of Sorcin, another RyR-interacting protein. Sorcin is a ubiquitously expressed 22 kDa Ca^{2+} binding protein that inhibits Ca^{2+} release via the RyR. This inhibitory effect is lost following its phosphorylation by PKA (Lokuta et al. 1997).

A cAMP phosphodiesterase (PDE) has also been identified within the RyR macromolecular complex (specifically, the PDE4D3 isoform). The presence of this enzyme provides a mechanism to tightly regulate cAMP, and thus PKA activity, in the vicinity of the receptor. The levels of this PDE isoform have been reported to be reduced in failing hearts (Lehnart et al. 2005).

Type 2 RyRs are associated with phosphatases to mediate rapid receptor dephosphorylation and return it to basal levels of activity. For example, calcineurin is suggested to be an accessory protein of cardiac RyRs (Bandyopadhyay et al. 2000), and is proposed to decrease Ca^{2+} channel activity. In addition, the phosphatases PP1 and PP2A are associated with cardiac RyRs (Marx et al. 2001). PP1 associates with RyRs via an interaction with spinophilin, whereas PP2A binds to a targeting protein PR130, which then anchors it to RyRs (Marx et al. 2001).

CARDIAC MYOCYTE CONTRACTION

Contraction of cardiac myocytes is facilitated by myofilaments organized into sarcomeres, situated along the long axis of the cell. The sarcomere consists of myosin-containing thick filaments surrounded by a hexagonal array of thin filaments, which are made up of actin polymers and troponin/α-tropomyosin (Tn/Tm) regulatory units (Parmacek and Solaro 2004). Additionally, each thin filament is separated at the Z-line by an actin binding protein, α-actinin.

Every seventh actin monomer comprising the thin filament is bound to a Tn/Tm complex. Tn is composed of three subunits: TnC, Troponin I (TnI), and Troponin T (TnT) (Greaser and Gergely 1971). TnC has a similar structure to CaM, and contains Ca^{2+} binding EF-hands (Parmacek and Solaro 2004). TnI is an inhibitory subunit, and TnT constitutively interacts with Tm. Binding of Ca^{2+} to TnC causes a conformational change in associated TnI. This enables Tn/Tm to slide into the groove between actin monomers, allowing the myosin thick filament to bind actin, thus forming a cross-bridge. By repetitive, transient actin-myosin interactions and utilizing energy from ATP

hydrolysis, the two filaments slide relative to each other, thus shortening the cell. Coordinated shortening of the entire myocyte population by the spreading AP leads to cardiac contraction. As cytosolic Ca^{2+} levels decline, Ca^{2+} is released from TnC leading to cross-bridge detachment, and the thick and thin filaments slide past each other back to their original positions. Cross-bridges cannot form between the filaments as they travel past in that direction.

Troponin proteins are regulated by phosphorylation, which alters their activity and thus affects cardiac myocyte contraction. PKA phosphorylation downstream from β-adrenergic stimulation is of particular importance, as this would facilitate altered cardiac contraction during, for example, physical exertion. Much work has been completed in elucidating the functional outcome of this modification, and the consensus of opinion is that PKA phosphorylation leads to increased cardiac contraction (for a review see Metzger and Westfall 2004). PKC phosphorylates regions on TnT and TnI. Early studies reported divergent results regarding the effects of PKC phosphorylation, although more recent studies point toward an inhibition of contractile function following PKC phosphorylation (Takeishi et al. 1998; Sumandea et al. 2004).

A large number of mutations have been identified in Ca^{2+}-dependent contractile proteins that are linked with specific cardiomyopathies (reviewed in Morimoto 2008). Clinically, cardiomyopathies can be divided into four main groups: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C). HCM is characterized by thickening of the ventricular walls with accompanying decreases in ventricular chamber volume. In this condition, systolic function is preserved at the expense of diastolic function, which is responsible for symptoms of heart failure and death in these patients. Thickened ventricular walls are also observed in DCM, in addition to an increase in chamber volume. The clinical outcomes include systolic dysfunction, which also leads to heart failure and sudden cardiac death. RCM is linked to diastolic dysfunction, although there is little or no effect on systolic function or ventricular wall thickness. Mutations leading to all three of these myopathies have been identified in the contractile proteins of the sarcomere, although interestingly none have yet been found that are linked to ARVD/C (Morimoto 2008). Considering TnT, for example, 27 mutations have been identified that have been linked to HCM, which act by increasing the Ca^{2+} sensitivity of cardiac muscle contraction. Two TnT mutations have been linked to DCM, the functional consequence being decreased Ca^{2+} sensitivity and an increased affinity of TnT for tropomyosin. Thirty-three mutations in TnI have been found that are linked with HCM, DCM, and RCM, mainly causing increased Ca^{2+} sensitivity and impaired interaction of TnT with TnI. Regarding TnC, a HCM-causing mutation has been identified that abolishes its interaction with TnI, thus losing the altered Ca^{2+} sensitivity imparted by PKA-dependent phosphorylation. Thirteen tropomyosin mutations have been identified, leading to HCM and DCM in the manner described previously.

Ca^{2+} EFFLUX

EC-coupling events are short-lived—atrial and ventricular myocytes reach peak contraction within a few tens of milliseconds of action potential initiation at the SA node. After cytosolic Ca^{2+} has activated the contractile units, it is rapidly extruded from the cytosol in preparation for the following action potential (Shannon and Bers 2004). The main efflux mechanisms in cardiac myocytes are the plasma membrane NCX and the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump on the SR. The plasma membrane Ca^{2+} ATPase (PMCA) and the mitochondrial uniporter may also play a more minor role. The relative contribution of these mechanisms varies in a species-dependent manner, for example in rabbit ventricles ~70% of cytosolic Ca^{2+} is removed by SERCA2a, 28% by NCX, and the remaining 2% by the mitochondrial uniporter.

A similar pattern is apparent in ventricles from dog, cat, guinea pig, and human. Alternately, in mouse and rat ventricles, SERCA2a removes ~92% of the Ca^{2+}, leaving only ~7% for the NCX (Bassani et al. 1994; Bers 2001). The difference in the relative contributions of the Ca^{2+} clearance pathways is reflected by the observed kinetics of NCX activity between different species (Sham et al. 1995). NCX current density is lowest in myocytes from rat, and highest in those from guinea pig. The adaptability of this system was shown in a recent report showing minimal cardiac dysfunction in a cardiac-specific SERCA2 KO mouse (Andersson et al. 2009). Ca^{2+} fluxes were maintained by increased activity of the L-type Ca^{2+} channel and NCX, demonstrating that these proteins can compensate for a major reduction in SERCA levels (<5% SERCA2 protein remained in myocardial tissue 4 weeks after gene excision).

In its forward mode, NCX uses the electrochemical gradient across the sarcolemma to translocate three Na^+ ions into the cytosol and expel one Ca^{2+}. In this situation, NCX is a depolarizing current. If Na^+ levels are high, the exchanger may change to its reverse mode, and bring Ca^{2+} into the cell. The exchanger was discovered around 40 years ago in the squid giant axon (Baker et al. 1969) and in the mammalian heart (Reuter and Seitz 1968). Following cloning of the canine NCX (Nicoll et al. 1990), three isoforms were identified (NCX1-3). Of these, NCX1 (110 kDa) is predominant in cardiac myocytes. It is responsible for exporting an amount of Ca^{2+} approximately equivalent to the flux entering via L-type Ca^{2+} channels (Bridge et al. 1990). The adaptability of cardiac myocytes is again shown in the NCX KO mouse, in which EC-coupling is maintained by a compensatory reduction in Ca^{2+} influx (Pott et al. 2007).

Cardiac NCX is regulated by the concentration of cytoplasmic ions, specifically activation by Ca^{2+} (Hilgemann 1990) and inhibition by Na^+ (Hilgemann et al. 1992). Maintenance of the ionic gradient between the extracellular space and cytosol is critical for NCX activity. In particular, intracellular Na^+ must remain low for NCX activity. This is facilitated by the action of the sodium potassium ATPase, which extrudes Na^+ entering the cell during the action potential. Inhibition of this pump by cardiac glycosides such as digoxin and ouabain results in an accumulation of intracellular Na^+, leading to a suppression of NCX activity and thereby attenuating Ca^{2+} efflux. As a consequence, Ca^{2+} transient amplitude and myocyte contraction are increased. Because of these positive inotropic effects, digoxin has long been used as therapy for failing heart. However, this manipulation can also lead to arrhythmia and myocyte death.

NCX has been shown to be phosphorylated by PKC and PKA suggesting that its activity may be regulated by these posttranslational modifications (Iwamoto et al. 1996; Ruknudin et al. 2000). NCX interacts with a number of accessory proteins. In particular, it interacts with the transmembrane protein phospholemman, which exerts an inhibitory effect (Zhang et al. 2003; Ahlers et al. 2005; Cheung et al. 2007). Phosphorylation of phospholemman is reported to occur on residues within the cytoplasmic carboxyl terminal of the protein, specifically Ser-68, and acts to inhibit NCX (Song et al. 2005a; Zhang et al. 2006; Cheung et al. 2007). This appears to be mediated by PKC and not PKA (Zhang et al. 2006). NCX has been identified within a macromolecular complex containing PKA and its anchoring protein mAKAP, together with PKC and the phosphatases PP1 and PP2A (Schulze et al. 2003), providing a robust mechanism for the phosphorylation and regulation of NCX and/or phospholemman.

Ca^{2+} reuptake into the SR is mediated by the SERCA pump, which uses energy from ATP hydrolysis. Molecular cloning has identified three SERCA isoforms (SERCA1–3), all of which undergo alternative splicing. The alternately spliced isoforms of SERCA2 (SERCA2a and SERCA2b) possess distinct carboxyl terminal residues. SERCA isoforms differ in their relative affinity for Ca^{2+} and their transport rates (Lytton et al. 1992). SERCA2a, the main cardiac isoform, possesses a $K_{1/2}$ of ~0.4 μM; however, in vivo, this is ~0.9 μM because of

its association with its regulatory transmembrane phosphoprotein, phospholamban (PLB). Cardiac SERCA pumps are usually situated on a region of the SR separate from the junctional zone where the RyRs are located. During recovery of a Ca^{2+} transient, Ca^{2+} is pumped into the SR at the location of the SERCA enzymes. The Ca^{2+} ions return to the dyadic SR lumen by tunneling through the SR network.

SERCA regulation in the heart is primarily governed by PLB. Inactive, phosphorylated PLB exists as a pentamer. It depolymerizes when not phosphorylated and can then interact with SERCA (Kimura et al. 1997). Unphosphorylated PLB monomers decrease the affinity of SERCA for Ca^{2+} (Tada et al. 1974). Subsequent phosphorylation of PLB prevents its interaction with SERCA, increasing the apparent activity of the pump and the rate of Ca^{2+} accumulation within the SR. PLB can be phosphorylated on three sites: Ser-16 by PKA, Thr-17 by CaMKII, and Ser-10 by PKC (Movsesian et al. 1984; Simmerman et al. 1986), facilitating regulation by a variety of signaling pathways. CaMKII is present in a multiprotein complex with SERCA2a, and can directly phosphorylate the Ca^{2+} pump (Toyofuku et al. 1994; Narayanan and Xu 1997) leading to enhanced activity (Xu and Narayanan 1999; Xu et al. 1999). Modulation of PLB-mediated SERCA inhibition is a major mechanism for acute enhancement of cardiac function following β-adrenergic receptor activation. As a result of reduced PLB interaction with the pump, the rates of clearance of the Ca^{2+} transient and relaxation is increased (positive lusitropic response), and SR store loading is enhanced. Because of greater Ca^{2+} within the store, the magnitude of Ca^{2+} fluxes is elevated thereby producing enhanced contraction.

SUBCELLULAR ORGANIZATION OF CARDIAC MYOCYTES

As described previously, the action potential generated at the SA node sweeps rapidly through the heart, coordinating cardiac contraction by activating atrial and then ventricular myocytes in synchrony. Within individual myocytes, the depolarization signal culminates in activation of the sarcomeric contractile units by elevating intracellular Ca^{2+}. The spatial properties of the Ca^{2+} increase depending on the structure of the different myocytes within the heart. For example, adult ventricular myocytes possess numerous invaginations of the sarcolemma, which form a regular array of inwardly directed membranous structures known as transverse tubules (T-tubule) (Fig. 1) (Song et al. 2005b). T-tubules are narrow (~200 nm diameter) and occur at regular intervals of ~2 μm (Brette and Orchard 2003). Additional branches project from the main T-tubules to give a complex network of sarcolemmal intrusions (Ayettey and Navaratnam 1978). T-tubules are a feature of mammalian ventricular myocytes, and are absent in the ventricles of birds (Bossen et al. 1978), reptiles, and amphibians (Bossen and Sommer 1984).

The presence of T-tubules facilitates homogenous Ca^{2+} transients during EC-coupling in ventricular myocytes. T-tubules serve to create dyadic junctions deep within the volume of a ventricular myocyte. In this way, Ca^{2+} sparks can be triggered simultaneously throughout a cell. The alternative to T-tubules is observed in neonatal myocytes and atrial myocytes, where dyadic junctions occur solely at the periphery of the cells (Fig. 2). T-tubules also act as important scaffolding regions for many of the proteins essential for Ca^{2+} signaling (Chase and Orchard 2011). For example, the sarcolemmal L-type Ca^{2+} channels and NCX are abundant on T-tubule membranes (Orchard and Brette 2008). It has been estimated that >75% of I_{Ca} flows into a myocyte through the T-tubules because of the high concentration of L-type Ca^{2+} channels in this region. As mentioned in the previous section, Ca^{2+}-dependent inactivation of I_{Ca} is one of the main mechanisms to curtail Ca^{2+} influx. This feedback mechanism appears to be more potent at T-tubules, meaning I_{Ca} at T-tubules is large but inactivates rapidly. In contrast, inactivation of I_{Ca} at the peripheral sarcolemma is slower overall, resulting in more Ca^{2+} entering the cell across the outer region of a cell than at T-tubule sites. This prolonged Ca^{2+} entry occurs during the

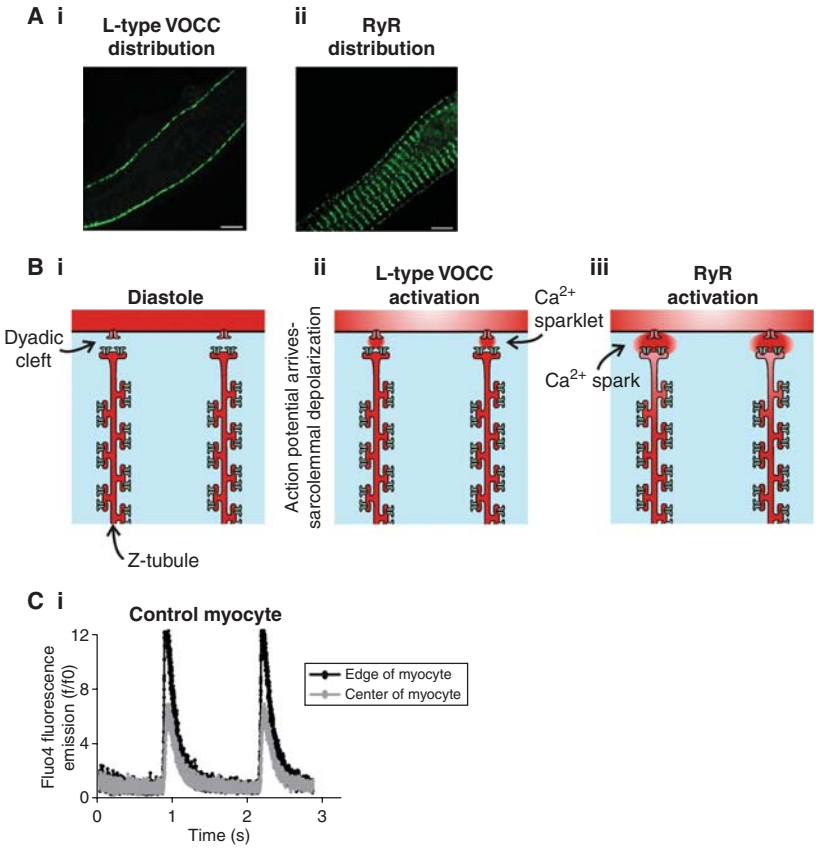

Figure 2. Excitation contraction coupling in atrial myocytes. Panel *A* illustrates the distribution of L-type VOCCs (*Ai*) and type 2 RyRs (*Aii*) in a section of an atrial myocyte. The pattern of L-type VOCC expression is clearly different from that in ventricular cells. The distribution of RyRs is similar to that in ventricular cells, except that there is an evident population of peripheral RyRs around the edge of the myocyte. Solely these peripheral RyRs align with the L-type VOCCs to produce functional dyads. Panel *B* is a cartoon sequence of events leading to the generation of a Ca^{2+} signal within an atrial myocyte. A small section of an atrial myocyte is depicted. There are no T-tubules, but instead two prominent SR tubules with a spacing of ~1.8 μm. Such SR tubules have previously denoted as "Z-tubules," as they occupy the Z-line (just like T-tubules). During the diastolic phase (*Bi*), the L-type VOCCs (red channels on the T-tubule membranes) and RyRs (blue channels on SR membrane) are silent. Arrival of the action potential causes depolarization of the sarcolemma and activation of the L-type VOCCs thereby generating "Ca^{2+} sparklets" at the periphery of the cell (*Bii*). The Ca^{2+} sparklets trigger activation of nearby RyRs thereby producing "Ca^{2+} sparks" (*Biii*). Panel *Ci* depicts the gradient of Ca^{2+} typically observed during electrical pacing of atrial myocytes. The Ca^{2+} signal at the edge of the cell (black trace) is larger and more rapidly rising than the central response (gray trace). The extent to which the Ca^{2+} signal occurs in the center of the cell (and thereby causes contraction) is dependent on the inotropic status of the cell. Application of a β-adrenergic agonist can make atrial Ca^{2+} signals become homogenous.

latter stages of I_{Ca}, and it has been proposed to promote SR Ca^{2+} loading in preparation for the following next cycle of EC-coupling.

Cardiac myocytes display an important homeostatic principle known as "autoregulation" (Eisner et al. 1998). This is essentially a balance between I_{Ca}, Ca^{2+} release from the SR and Ca^{2+} efflux via NCX. Acute changes in any one of these fluxes will exert compensatory changes in the others that bring systolic Ca^{2+} transients back to normal levels. For example, under conditions of increased SR, Ca^{2+} release

following RyR phosphorylation Ca^{2+} influx through I_{Ca} is reduced and Ca^{2+} efflux via NCX is increased, thus maintaining the steady-state systolic Ca^{2+} transient. Taking into consideration that Ca^{2+}-dependent inactivation following SR Ca^{2+} release occurs predominantly at the T-tubules, and NCX is also concentrated in this region, it is evident that autoregulation occurs mainly at the T-tubules. Because of the principle of autoregulation, prolonged changes in inotropic status require sustained changes in more than one component of the I_{Ca}/Ca^{2+} release/NCX triumvirate (Eisner et al. 2009).

The T-tubular network of atrial myocytes is generally not as well developed as that observed in ventricular myocytes, especially in the atrial myocytes of small mammals. The sarcolemmal L-type Ca^{2+} channels of atrial myocytes provide a triggering Ca^{2+} signal for a small population of "junctional" RyRs situated below the sarcolemma at the periphery of the cells (Bootman et al. 2006). The consequence of this arrangement is that the initial Ca^{2+} influx (I_{Ca}) activates Ca^{2+} sparks solely from the peripheral SR (Figs. 1 and 2). In the absence of a positive inotropic agonist, the Ca^{2+} signal remains confined to the cellular periphery and contraction is minimal (Bootman et al. 2011).

In addition to the junctional RyRs, atrial myocytes express a major population of nonjunctional RyRs that form a 3-dimensional lattice of Ca^{2+} release sites within the cells (the distribution of RyRs is actually very similar between ventricular and atrial myocytes—the location of L-type VOCCs is different) (Figs. 1 and 2) (Chen-Izu et al. 2006; Schulson et al. 2011). Because these nonjunctional RyRs are not located within a dyadic junction, they are not activated by I_{Ca} (Mackenzie et al. 2001). However, they can be activated by CICR if the peripheral Ca^{2+} signal is sufficient to act as a trigger for a Ca^{2+} wave. The depth that such centripetal Ca^{2+} waves propagate within an atrial myocyte determines the extent of contraction—the deeper a Ca^{2+} wave spreads the more contractile filaments will be engaged (Mackenzie et al. 2004). The extent of atrial myocyte contraction is regulated by controlling the spread of the centripetal Ca^{2+} waves. The atrial myocytes in some mammalian species display a relatively high degree of T-tubule membrane. Why some atrial cells should rely on T-tubules when others do not is unclear. However, it has been shown that the presence of T-tubules within individual atrial myocytes is correlated with cell diameter, suggesting that tubulation acts to coordinate Ca^{2+} signaling in larger cells (Smyrnias et al. 2010).

Neonatal rat ventricular myocytes are similar to atrial cells in that they lack a fully formed T-tubule network. The T-tubules appear progressively through development (Sedarat et al. 2000). Just as with atrial myocytes, the main region of VOCC-RyR interaction within a neonatal myocyte is at the periphery of the cell. Therefore, Ca^{2+} signals within a neonatal myocyte closely resemble those in an adult atrial cell, and change to become homogenous Ca^{2+} signals when T-tubules arise.

Ventricular myocytes from hearts that are progressing toward failure show decreased organization of their T-tubules, and loss of T-tubules (Fig. 3) (He et al. 2001). As a result, there is reduced coupling efficiency between the L-type VOCCs on the sarcolemma and RyRs on the underlying SR. The loss of T-tubule membranes means that dyadic junctions are lost and RyRs become "orphaned." This leads to abnormal EC-coupling characterized by an increased propensity for arrhythmia and decreased magnitude of the Ca^{2+} response and contraction (Louch et al. 2004). EC-coupling in detubulated ventricular myocytes resembles that observed in atrial myocytes, whereby Ca^{2+} signals are initiated at the plasma membrane and propagate through the myocyte by the relatively slow process of CICR (Smyrnias et al. 2010).

ADAPTATION TO DISEASE

In response to pressure or volume overload, damage, or genetic factors, the heart mounts an adaptive hypertrophic response. Because cardiac myocytes are terminally differentiated, enlargement of the heart results mainly from growth of existing myocytes and not as a result

of proliferation. For many hypertrophic stimuli, the remodeling of the heart is initially beneficial by acting to increase cardiac output. However, under conditions in which stress persists, for example, because of hypertension, the remodeled heart undergoes a process known as "decompensation." Specifically, the thickness of the ventricle wall diminishes, and the ability of the heart to supply the cardiovascular requirements of the organism is lost. Such pathological cardiac hypertrophy has a poor prognosis and leads to cardiac failure and sudden death (Levy et al. 1990).

A widely supported hypothesis is that changes in Ca^{2+} cycling mediate the hypertrophic response (Molkentin 2006). Although initial modifications of Ca^{2+} handling are beneficial, as the heart progresses to failure they may contribute to pathology (Fig. 3) (Roderick et al. 2007). A general feature of calcium fluxes during adaptive hypertrophy is an increase in the amplitudes of the Ca^{2+} transient, and also of the Ca^{2+} sparks that underlie them. Contributing to this is an increase in SR store loading mediated by increased SERCA pump activity. SERCA activity is enhanced through several mechanisms: increased SERCA expression, decreased PLB expression, and increased PLB phosphorylation. A decrease in NCX current and an increase in RyR activity have also been detected during this stage of hypertrophy.

As the heart progresses to failure, further changes in Ca^{2+} regulation and flux are observed. Contributing to the decreased contractility of the failing heart is a general decrease in the amplitude of each action potential-evoked Ca^{2+} transient. Underlying this reduced Ca^{2+} signal are a number of factors, foremost of which is a decrease in SERCA activity (Fig. 3) (Hoshijima et al. 2006). SERCA activity may be modified through a reduction in its expression, increased PLB expression or decreased PLB phosphorylation. As a result of this decrease in SERCA function, diastolic Ca^{2+} is elevated (a common feature of the failing heart) while myocyte relaxation is prolonged and SR store content is diminished (Roderick et al. 2007). The key contribution of SERCA dysfunction to the decrease in cardiac function during failure has led to the development of viral-mediated SERCA overexpression for gene therapy (Lyon et al. 2011).

Failing hearts are also characterized by more arrhythmic Ca^{2+} signals. This is perhaps surprising given that SR store loading—a key determinant of RyR activity—is decreased. RyRs isolated from failing hearts and incorporated into lipid bilayers are more spontaneously active than RyR isolated from control hearts, perhaps explaining this conundrum (Kubalova et al. 2005). Indeed, myocytes from failing hearts showed decreased store loading, increased spontaneous RyR opening (observed as Ca^{2+} sparks) and decreased SR Ca^{2+} reuptake. Another mechanism for increased arrhythmic Ca^{2+} signaling during heart failure is the expression of inositol 1,4,5-trisphosphate ($InsP_3$) gated Ca^{2+} release channels ($InsP_3Rs$). $InsP_3Rs$ are typically >50-fold less abundant than RyR in healthy myocytes (Kockskamper et al. 2008). However, their expression increases significantly during hypertrophy and heart failure. In particular, their expression within dyadic junctions increases (Fig. 3) (Harzheim et al. 2009, 2010). The up-regulation of $InsP_3Rs$ provides a positive-feedback loop that can promote further hypertrophic remodeling (Nakayama et al. 2010). Although $InsP_3Rs$ are not capable of mounting significant Ca^{2+} signals by themselves, their location next to RyRs within dyadic junctions means that they can trigger CICR and thereby augment EC-coupling. The expression of $InsP_3Rs$ may be an initially beneficial adaptive aspect of myocyte remodeling in that they can help to increase systolic Ca^{2+} transients and evoke greater contraction. However, concomitant with this potentially beneficial aspect of enhanced $InsP_3R$ expression is an undesirable increase in the propensity of cells to show spontaneous Ca^{2+} release events that may cause arrhythmia. This is because of the fact that $InsP_3Rs$ are not solely tuned to the activation of I_{Ca}, but can open independently whenever cytosolic $InsP_3$ levels are sufficient (Harzheim et al. 2009).

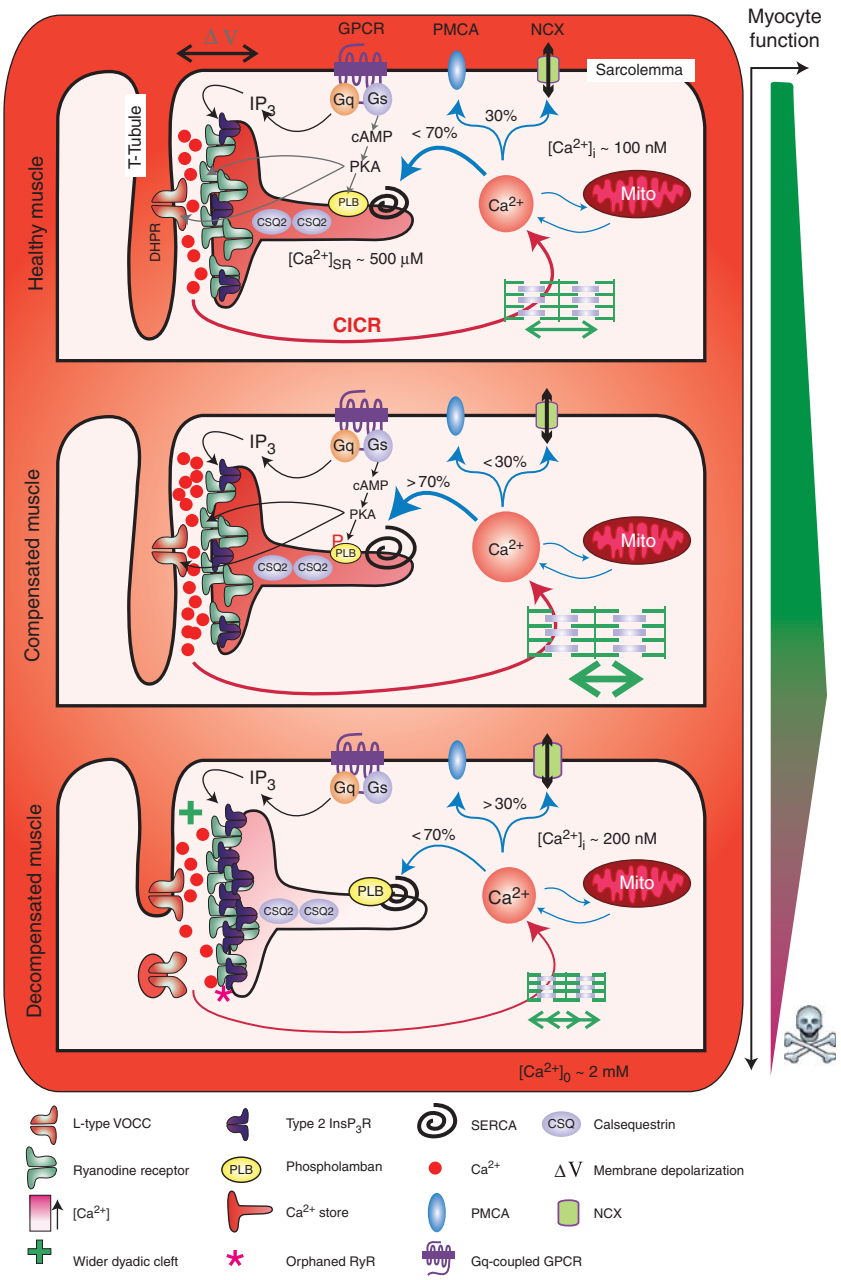

Figure 3. Excitation contraction coupling in healthy and decompensated hypertrophic cardiac muscle. The figure depicts the architecture and molecular composition of a cardiac dyad in a normal, healthy myocyte (*upper* panel), a compensated hypertrophic situation (*middle* panel), and a decompensated failing myocyte (*lower* panel). A key depicting the symbols used to represent the major players in EC-coupling is provided at the *bottom* of the figure. Depolarization of the plasma membrane results in Ca^{2+} influx through L-type voltage gated channels in the T-Tubule, which stimulates Ca^{2+} release via RyRs located on the juxtaposed SR. Following diffusion out of the dyadic cleft, Ca^{2+} encounters the contractile filaments causing myocyte contraction. Myocyte relaxation is then brought about by Ca^{2+} recycling back into the SR by the SERCA pump or extrusion across the plasma membrane via NCX. (*See facing page for legend.*)

The importance of cellular architecture to EC-coupling is highlighted by its contribution to Ca^{2+} dysregulation during cardiac failure. In myocytes from failing heart, the synchronicity of the initiation of the Ca^{2+} transient is reduced and the amplitude of the transient diminished (Gomez et al. 1997). Work from a number of laboratories using live and fixed cell staining approaches, have now established that T-tubules are lost or atrophied as hypertrophy develops, whereas the distribution of RyRs on the SR is unaffected (Gomez et al. 2001; Song et al. 2005b). As a consequence of this membrane remodeling, RyRs become progressively orphaned. Deficiencies in EC-coupling because of T-tubule remodeling have also been observed during the earlier stages of hypertrophy, albeit with less dramatic consequences (Xu et al. 2007). During this stage, the width of the dyadic cleft is marginally increased, altering the kinetic properties of coupling between membrane depolarization and Ca^{2+} release from the SR. Inotropic stimuli such as adrenaline, which serve to increase Ca^{2+} influx and sensitivity of RyRs to release, overcome this deficiency in EC-coupling thus indicating that the Ca^{2+} signaling machinery is still intact. Similarly, dyadic $InsP_3Rs$, which as indicated earlier are elevated during hypertrophy, may also contribute to overcoming the decreased efficiency of coupling.

CONCLUSION

The raison d'etre of cardiac myocytes is controlled contraction in response to repetitive electrical depolarization signals—a function that is fundamentally controlled by Ca^{2+}. Although there are a limited number of key players—I_{Ca}, NCX, SERCA, RyRs, TnC—involved in generating and reversing Ca^{2+} signals, they are subject to numerous levels of regulation and an array of interactions with other proteins. Furthermore, the cellular location of the Ca^{2+} signaling systems is critical in determining the spatial properties of the Ca^{2+} signals during EC-coupling. Cardiac Ca^{2+} signals can be acutely altered to provide rapid changes in cardiac output. The heart responds to long-term increased hemodynamic demand by remodeling. This remodeling encompasses both structural changes and altered gene expression. Depending on the stimulus, this can be an adaptive, reversible remodeling that promotes Ca^{2+} signaling and cardiac function. Alternatively, a heart can become committed to an

Figure 3. (*Continued*) Neurohormonal activation of $G\alpha_q$-coupled receptors leads to the formation of $InsP_3$, which stimulates Ca^{2+} release via $InsP_3Rs$ located in the dyad. This $InsP_3$-stimulated Ca^{2+} release sensitizes neighboring RyRs, causing enhanced Ca^{2+} fluxes and increasing the frequency of arrhythmic events. Activation of β-adrenergic receptors increases intracellular cAMP, which activates PKA leading to phosphorylation of PLB. On phosphorylation, PLB dissociates from SERCA thereby enhancing Ca^{2+} transport activity. Compensated/adaptive hypertrophy is associated with enhanced EC-coupling. Significantly contributing to this phenotype is an increase in SR store loading. This is brought about by an up-regulation of SERCA activity mediated by either an increase in SERCA or decrease in PLB expression. Alternatively, as a result of PKA-dependent phosphorylation, PLB interaction with SERCA and suppression of Ca^{2+} transport may also be decreased. Increased SERCA activity also serves to increase the rate of relaxation thereby allowing more rapid cycles of myocyte contraction. The width of the dyadic cleft may also marginally increase at this stage of hypertrophic remodeling. However, IP_3Rs are up-regulated in the dyad during hypertrophy supplementing the Ca^{2+} signal arising via RyRs to possibly further support EC-coupling. During decompensated hypertrophy, myocyte architecture and protein expression are remodeled. Specifically, the width of the dyadic cleft is increased making it harder for Ca^{2+} arising via VOCCs to activate Ca^{2+} release from RyRs. T-Tubules also atrophy resulting in orphaned RyRs. The SERCA-PLB ratio is also modified to favor decreased SERCA activity. Notably, $InsP_3R$ expression in the dyad is increased. As a result, more of the RyRs that are located in this region are close enough to $InsP_3Rs$ to be affected by Ca^{2+} arising from them. Overactivation of these $InsP_3Rs$, for example, by the elevated levels of circulating ET-1 present during heart failure, promotes arrhythmias thereby contributing to the pathology associated with heart failure.

irreversible form of remodeling that causes progressively weaker and more arrhythmic Ca^{2+} signaling. The remodeling causes changes in Ca^{2+} signaling and vice versa. Therefore, within the context of the heart Ca^{2+} is very much a signal for both life and death.

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